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MICROSCOPIC STUDIES

ON THE

CENTRAL NERVOUS SYSTEM

OF

Reptiles and Batrachians.

THE SPINAL CORD OF THE FROG—RANA PIPIENS, RANA HALECINA.

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Microscopic Studies on the Central Nervous System of Reptiles and Batrachians.

By John J. Mason, M. D.

ARTICLE I.—THE SPINAL CORD OF THE FROG—RANA PIPIENS, RANA HALECINA.

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IT is not intended in these articles to give, in detail, an anatomical description of the nervous system of this class of animals. So far as the anourous group of batrachians is concerned, one could hardly effect such a purpose better than by translating the works of either Reissner * or Stieda,† which together with those of Wyman‡ and Ecker § are in the hands of most comparative anatomists. Only in writing of species, the nervous system of which may not previously have been studied, will a full description be attempted, the main object being to present from time to time facts observed by the author, and regarded by him as supplementary. The form of the spinal cord and especially that of its enlargements; the nuclei of the nerve cells, and variations in their shape, size, etc., in the same individual; the number of ganglionic bodies in the spinal cord, and their relations to the roots of the spinal nerves, and the differences, if any, which may be determined by sex: these, among others, seem to me to be subjects of

Der Bau des Centralen Nervensystems der Ungeschwänzten Batrachier. Dorpat, 1864.

†*Studien ueber das Central Nervensystem der Wirbelthiere.* Leipzig, 1870.

‡*Anatomy of the Nervous System of Rana pipiens.* Washington, 1853.

§*Icones Physiologicæ,* 1851–59. Liepzig. "Die Anatomie des Frosches des physiologischen Thieres, ist fur den Physiologen, kaum minder wichtig, als die Anatomie des Menschen."

much interest, many of which can be examined remarkably well in cold-blooded animals.

Before describing the method of making preparations which I employ, a few features of the process of Stieda will be noticed. This observer places the entire brains of small animals, first in a solution of 80–90 per cent. alcohol, which has been tinted yellow by iodine. As soon as the piece feels firm to the touch (one to four days according to the size of the specimen) it is placed in a dark yellow solution of bichromate of potash, care being taken to use a large excess of the solution. After a time varying from three weeks to three months, the hardened part is placed in strong ammoniacal carmine, and is removed after from one to five days, placed in alcohol, and after all excess of carmine has been removed, is ready for section.

By this process Stieda has been able to prepare sections in a long unbroken series, of the brains of mice, frogs, etc., saving thereby the risks of several transfers.

My own method is the old one, except in regard to the solutions for hardening. A two per cent. solution of bichromate of potash has given me excellent preparations, also the solution of Clarke, which consists of a solution (1–800) of chromic acid, to each ounce of which a grain of bichromate of potash is added. So far I prefer to stain after cutting. At least two transfers may be avoided by using a siphon tube, to remove alcohol or water, and with the flattened spoons of Seguin, there is but little danger of injuring the sections. After the piece has remained from three to five weeks in the two per cent. bichromate solution, which it is well to renew every two weeks at least, it is placed in Ivanoff's modification of Müller's fluid, which consists of one or more parts of sulphate of soda, until ready for section, when it is transferred to alcohol for a few minutes.

The membranes of the cord ought not to be removed until just before making the sections, or until the part is thoroughly hardened, otherwise deformity will surely result, showing itself in an oval instead of circular central canal. When an unbroken series is not desired, it is better to make sections with the membranes on. After cutting, the sec-

tions are stained in Beale's glycerine and water solution of ammoniacal carmine, for which I often substitute the borax carmine of J. W. S. Arnold, after adding to it some ammonia, which rather improves the color. Transparency is effected by oil of cloves, after absolute alcohol has expelled the water, and the mounting is done in Canada balsam dissolved in chloroform. A short piece of copper wire hammered flat at one end and bent at right angles is a good substitute for the steel spoon, as it can be quickly made of any size, and its outline changed by scissors when desired.

The sections of the alligator's spinal cord, which were shown with photo-micrographs at the last annual meeting of the American Neurological Association, measured about 9–10 mm. through the brachial and crural enlargements, and were made from specimens hardened by the same process as that recommended by Seguin* for the human cord.

To prepare isolated nerve-cells, there is perhaps no better method than that employed by Karabanowitsch, viz.: maceration for forty-eight hours in a weak solution of bichromate of potash (2–100) mixed, equal parts, with a (1–100) solution of caustic soda and ammoniacal carmine.

It is possible, however, to make beautiful preparations of the nerve-cells of the frog, by simple agitation, with some teasing, in a drop from a solution of glycerine, water and carmine. Agitation in osmic acid is another common means of isolation, but the preceding methods are generally to be preferred.

The large cellular structures which form such a prominent group in the inferior horns of grey matter are composed of large, sharply defined nuclei, surrounded by protoplasmic masses which, under the action of certain re-agents, look as if they were composed of fibrillæ, which unite in bundles to form what are called the cell processes. The nuclei seem to be but slightly affected by these re-agents, but by prolonged action, seem to be compressed by the surrounding mass. In the cells of medium size the nuclei are rarely if ever changed from the spherical form or circular appearance. These nuclei which contain a distinct nucleolus, are not too numerous to be counted in the frog. For example, in an unbroken series of

*Stricker's *Handbook*, p. 646.

twenty-four sections, from the middle of the brachial enlargement of Rana halecina, I counted in both inferior horns 540 large and medium-sized nuclei, and in an unbroken series of twenty-four sections from the middle of the crural enlargement I counted in both horns 390 nuclei. Reissner * estimated the length of the brachial enlargement as standing to that of the crural enlargement in the ratio of 6–10, and this proportion is true of the American species, as would be supposed. It seems fair to conclude therefore, that while the crural enlargement in frogs has a smaller transverse diameter than that of the brachial enlargement, still, by its greater length it contains as many if not more ganglion cells than the latter. It is then, perhaps, the larger of the two swellings and corresponds, as it ought, with the larger size of the posterior extremities.

The preponderance of the lumbar enlargement in birds, and the equality of the two which I have observed in the alligator† and in several species of lizards can undoubtedly be explained, by regarding the amount of grey matter, or possibly the number of nerve-cells, as the surer indication of importance of function in different regions of the cord.

The large crural nerve cells, as well as their nuclei, are larger than those of the brachial region.

This fact I have established by numerous measurements, which may be condensed into the following average diameters for Rana halecina: Brachial nuclei, long diameter, 7; crural nuclei, long diameter, 8; brachial nuclei, short diameter, 6.5; crural nuclei, short diameter, 7.5. The numbers denote divisions of the micrometer eye-piece, each division with the objective used representing .002 mm.

After a thorough comparison of sections from the brachial region of twenty large specimens of Rana pipiens, of which seven were males, I have been unable to detect any difference either in the arrangement, size or structure of the elements, that could reasonably be referred to as explaining the remark-

* Loc. cit.—Length of intumescentia anterior, 6 mm.; that of intumescentia posterior 10 mm.

† See "Transactions of American Neurological Association," in preceding number of the JOURNAL.

able and purely reflex energy displayed by the male frog during the embrace of copulation.* The group of cells with nuclei of medium size, described by me in the *New York Medical Journal*, of December last, is present in the "pars media" of cords from both sexes in three species which I have examined with especial reference to this point.

The distribution of the inferior root fibres in the inferior horns of grey matter, among and to the ganglion cells.

Wyman,† whose memoir appeared at the time Ecker was preparing his *Icones Physiologicæ* for publication, states, page 20: "After the most careful examination, I have not detected any direct connection between these caudate appendages and nerve tubes;" while the latter anatomist prints an illustration after Küpfer ‡ which represents, in a cross section made through the middle of the brachial enlargement, four large nerve cells, with processes running downwards and outwards, as far as the periphery, and two other large cells, from the same group, with processes continuous with the superior (posterior) roots!

Such an exaggerated illustration could never have been made by photography.

Reissner, § ten years later, in a work than which there are few more perfect memoirs, writes: "In regard to the inferior roots, I must premise, that the spinal cord of anourous batrachians, which have been at my disposal, is very little suited for observing the entrance and course of the fibres in the grey substance. * * * * As before stated, a nerve cell may lie near the point of entrance of a bundle, and send one of its processes into the same. (Fig. M.) * * * * The remainder of the nerve root fibres which run upwards or outwards in the grey matter, unite either wholly or in part with large nerve cells."

Stieda, ‖ after contending for the true cell structure of the protoplasmic mass which contains the nucleus, and the struc-

* Goltz.—*Beiträge zur Lehre von den Functionen der Nervencentren des Frosches.* Berlin, 1869.

† Loc. cit.

‡ *Diss. de Medullæ Spinalis Textura in Ranis.* Dorpat, 1854.

§ Loc. cit. pp. 19, 20.

‖ Loc. cit. p. 150, et seq.

tureless state of the axis cylinder, against the views of those who believe in their fibrillary composition, enquires: "How is it now with the connection between nerve-cells and nerve-fibres? * * * * From my observations on fresh as well as on hardened ganglia of the spinal nerves in fishes, amphibia, warm-blooded animals and man, the union is of this nature: the axis cylinder of the nerve fibre is the direct continuation of the cell substance;" and two pages further on, "It seems to me impossible to see such a connection in sections of the brain and spinal cord; it can only be done with the help of isolating methods, as we are taught by the latest observations of Koschewnikoff."* It will be observed that although Stieda has not seen such a connection in a section, he nevertheless considers it as a fact, while Reissner, in 1864, claimed to have seen it; and it was figured by Ecker and Küpfer. Dean † has also a plate showing, in the rabbit, a nerve process joining a nerve-fibre of an anterior root, and passing out as far as the periphery. The frequency with which I have obtained sections from the brachial enlargement of the frog, which show in the clearest manner what becomes of by far the greater part of the inferior root fibres of this region, leads me to give here my manner of procedure.

In the first place, I must assert, contrary to the opinion of Reissner given above, that I know of no other animal so well suited, and for so many reasons, as is the frog, for the study of this point. It is true that the root-fibres in each section are comparatively few; but this seems to me an advantage rather than an impediment, and if more nerve-fibres are desired, it is quite easy to obtain them by making more sections. The supply of frogs is ample; and their spinal cords almost come out of the spinal canal of themselves, so easy is their removal, when the operation is done upon the abdominal side. As the chief cause of failure lies in the division by the razor of the nerve-tube somewhere between the cell and the periphery of the cord, it follows that, *cæteris paribus*, the smaller the diameter of the cord, the greater the chance of success. In

* *Archiv für Microsk. Anat.* Bd. V. 1869.

† J. Dean.—*Microscopic Anatomy of the Lumbar Enlargement of the Spinal Cord.* 1861.

the brachial enlargement, many fibres from the inferior roots enter the cord at right angles, and remain in the same vertical plane while describing a curve laterally, the convexity of which lies towards the inferior median fissure. This is why longitudinal sections are useless for showing this connection; while a series of transverse sections, made in the section cutter, including the roots of the second pair of nerves, will often show, in well stained specimens, the greater portion, if not all, the root fibres passing through the white substance, and, after entering the grey substance, branching outwards among the cells. Most of the fibres lose themselves among the larger external group of cells, while a few are seen to unite with the upper group. In several instances I have traced a connection between cell process and nerve-fibre, with so much certainty, confirming the observation by using a binocular instrument, that I am forced to believe not only in the possibility, but the facility of demonstrating in this way an important fact.

It is always advisable to submit the transparent section to microscopic examination before it is covered, as the weight of the cover is often sufficient to sever fibres. Good objectives, of considerable power, can be used in studying uncovered preparations; and I have succeeded in obtaining satisfactory photographs of some of these.

CONCLUSIONS.

1. The central canal of the spinal cord of frogs is more nearly cylindrical in shape than has been generally supposed. The oval contour is not seen in cross sections below the second pair of nerves, when the membranes are not removed before hardening.

2. The nuclei of the large nerve-cells are more generally oval in form than are those of the smaller cells. I have confirmed this in a few fresh preparations only. It is possible that the re-agents employed have a different effect upon the two classes of nuclei, but it seems more reasonable to conclude that they have a different form anatomically.

3. The nerve-cells of the crural enlargement are as abundant as those of the brachial enlargement, if not more so. Their nuclei are larger, as are also the surrounding masses of protoplasm or cell bodies.

4. No difference in structure can be made out in the upper portion of the cord, corresponding with the sexual function in the male. The long-continued and violent tonic spasm of the anterior extremities, must be explained by local hyperæmia influencing the same structures as those which exist in the female.

5. The relation which is generally believed to exist between the so-called motor-cells and the inferior (anterior) roots, can be demonstrated in the frog more readily than in any other animal.